

The paragraph at p. 21, 2<sup>nd</sup> para., lines 8-12:

The PCR amplification products of the RLR 1, 2 and 3 reactions were purified with the WIZARD PCR PREPS system (PROMEGA) and eluted in 45 µl of buffer T. 6 µl of each purified PCR were incubated 1 hour at 37 °C in a mixture containing 3 µl of restriction buffer C, 3 µl of BSA (1 mg/ml), 20 U of the *Eco* RI enzyme, 10 U of the *Nco* I enzyme and 15 µl of water.

The paragraph at p. 21, 4<sup>th</sup> para., lines 20-23:

The linearized vectors as well as the digested PCR were purified on a TBE 1% agarose gel with the QIAquick system (QIAGEN). Each vector or each digested PCR was eluted in 30 µl of buffer T.

**IN THE CLAIMS:**

Please cancel claims 1-39.

Please add the following new claims:

40. A ligation-mediated *in vitro* method of recombining polynucleotides from a polynucleotide library, comprising:
- fragmenting polynucleotides from the library,
  - hybridizing the fragments to an assembly template,
  - ligating the hybridized fragments,
  - optionally denaturing the hybridized fragments from the template,
  - repeating the hybridizing step, before or after the ligating step or the denaturing step, as necessary until ends of the hybridized fragments are substantially adjacent to each other on the assembly template, and
  - ligating the adjacent ends to form a recombined polynucleotide.

41. The method of claim 40, wherein any polymerase used in the method is used only to amplify the recombined polynucleotide, to amplify polynucleotides from the library, or to amplify the fragments before the first hybridizing step.
42. The method of claim 40, further comprising adding assembly templates before completion of the hybridizing step(s).
43. The method of claim 40, wherein the polynucleotides from the library are double-stranded and these double-stranded polynucleotides or fragments thereof are denatured before the hybridizing step(s).
44. The method of claim 40, further comprising selecting the recombined polynucleotide that possesses advantageous properties.
45. A ligation-mediated *in vitro* method of recombining polynucleotides from a polynucleotide library, comprising:
  - fragmenting polynucleotides from the library,
  - hybridizing the fragments to an assembly template,
  - ligating the hybridized fragments,
  - denaturing the hybridized fragments from the template,
  - repeating the hybridizing step multiple times, before or after the ligating step or the denaturing step, until ends of the hybridized fragments become substantially adjacent to each other on the assembly template, and
  - ligating the adjacent ends to form a recombined polynucleotide.
46. The method of claim 45, wherein any polymerase used in the method is used only to amplify the recombined polynucleotide, to amplify polynucleotides from the library, or to amplify the fragments before the first hybridizing step.
47. The method of claim 45, further comprising adding assembly templates before completion of the hybridizing steps.

48. The method of claim 45, wherein the polynucleotides from the library are double-stranded and these double-stranded polynucleotides or fragments thereof are denatured before the first hybridizing step.
49. The method of claim 45, further comprising selecting the recombined polynucleotide that possesses advantageous properties.
50. A ligation-mediated *in vitro* method of recombining polynucleotides from a polynucleotide library, comprising:
- fragmenting polynucleotides from the library,
  - hybridizing the fragments to an assembly template,
  - ligating the hybridized fragments,
  - optionally denaturing the hybridized fragments from the assembly template, and
  - repeating the hybridizing step, before or after the ligating step or the denaturing step, as necessary to form a recombined polynucleotide,
- wherein any polymerase used in the method is used only to amplify the recombined polynucleotide, to amplify polynucleotides from the library, or to amplify the fragments before the first hybridizing step.
51. The method of claim 50, wherein, before the last ligating step, the ends of the hybridized fragments are substantially adjacent to each other on the assembly template.
52. The method of claim 50, further comprising adding assembly templates before completion of the hybridizing step(s).
53. The method of claim 50, wherein the polynucleotides from the library are double-stranded and these double-stranded polynucleotides or fragments thereof are denatured before the hybridizing step(s).
54. The method of claim 50, further comprising selecting the recombined polynucleotide that possesses advantageous properties.
55. The method of claim 44, further comprising amplifying the recombined polynucleotide before the selecting step.

56. The method of claim 44, further comprising separating the recombined polynucleotide from the assembly template before the selecting step.
57. The method of claim 56, further comprising cloning the recombined polynucleotide after the separating step and before the selecting step.
58. The method of claim 40, wherein the assembly template comprises oligonucleotides that are complementary to the 3' ends of some of the fragments and to the 5' ends of some of the other fragments.
59. The method of claim 40, wherein substantial portions of the library polynucleotides are homologous to each other or complementary to portions of the assembly template.
60. The method of claim 40, wherein substantial portions of the hybridized fragments are complementary to portions of the assembly template that are substantially adjacent to each other.
61. The method of claim 40, wherein the hybridizing and ligating steps are carried out simultaneously.
62. The method of claim 40, wherein the fragmenting is random.
63. The method of claim 62, wherein the random fragmenting comprises treatment with DNase I and wherein the library comprises partially heterologous double-stranded polynucleotides.
64. The method of claim 40, wherein a researcher using the method controls the fragmenting and chooses the degree of recombination, the points of recombination, or both.
65. The method of claim 64, wherein the fragmenting comprises hydrolyzing the polynucleotides from the library with restriction enzymes.
66. The method of claim 65, wherein the hydrolyzing is performed with several different restriction enzymes or with restriction enzymes that have a plurality of cutting sites on the polynucleotides from the library.

67. The method of claim 65, wherein the hydrolyzing comprises separately hydrolyzing polynucleotides from at least two distinct polynucleotide libraries by subjecting the distinct libraries to different restriction enzymes.
68. The method of claim 40, further comprising adding enzymes that recognize and cut non-hybridized ends of the hybridized fragments in a specific manner when the non-hybridized ends overlap other hybridized fragments on the same assembly template.
69. The method of claim 68, wherein the enzyme is a flap endonuclease.
70. The method of claim 68, wherein the ligating step(s) utilizes a ligase that is thermostable and active at high temperature.
71. The method of claim 70, wherein the enzymes that recognize and cut the non-hybridized ends have the same thermostability and high temperature activity as the ligase.
72. The method of claim 40, wherein some of the fragments serve as the assembly template for some of the other fragments.
73. The method of claim 40, wherein the library consists essentially of polynucleotides obtained by successive steps of directed mutagenesis, by error prone PCR, by random chemical mutagenesis, by *in vivo* random mutagenesis from a single gene, or by recombining genes of close or distinct families within the same or different species.
74. The method of claim 40, wherein the library comprises synthetic polynucleotides.
75. The method of claim 40, wherein, before the first hybridizing step, the template is double stranded.
76. A vector comprising the selected recombined polynucleotide of claim 44, 49 or 54.
77. A cellular host transformed by the selected recombined polynucleotide of claim 44, 49 or 54.
78. A protein encoded by the selected recombined polynucleotide of claim 44, 49 or 54.
79. A library formed from the recombined polynucleotide of claim 40, 45 or 50.

80. A library formed from the selected recombined polynucleotide of claim 44, 49 or 54.

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